On the Spermatogenesis of the Louse (Pediculus corporis and P. capitis), with some Observations on the Maturation of the Egg.

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With Plate 16 and 1 Text-figure.

1. Introduction.

THE work described in this paper was undertaken in the hope of throwing some light on certain curious phenomena with regard to sex which had occurred in breeding experiments carried on with the body- and head-louse by Hindle and by Bacot. Hindle (**6, 6a**) observed that among the offspring of single pairs of lice, although frequently both sexes appeared in about equal numbers, quite often the whole family reared consisted either only of males or only of females, or in other cases was almost entirely of one sex, with one or two offspring of the other. He also found that when a female, which, with one mate, had given a family of a certain type, was paired with a different mate, the family might be of another type, e. g. a female mated with male A gave only male offspring (fourteen ♂ ♂), but when mated with male B she gave both males and females (three ♀ ♀, one ♂). There were also indications that the same male paired with two different females might have offspring of different sexes.

The second remarkable observation in regard to sex was made by Keilin (**7**) on material reared by Bacot. Bacot crossed the male head-louse (P. capitis) with the female

body-louse (P. corporis), and on making an anatomical examination of some of these hybrids Keilin found that a number of them were analogous to the "intersexes" obtained by Goldschmidt (4, 5) in the hybrids between different races of the moth Lymantria (Liparis) dispar. These "intersexes" can hardly be called gynandromorphs—a name which implies a sharp division of the body into regions having male and female characters, but are rather an intimate blend or fine mosaic of male and female organs or characters. Since Goldschmidt has based very fundamental conclusions with regard to the physiology of sex-determination upon his study of the intersexes of L. dispar, and extremely few other instances of the kind are hitherto known for comparison, it seemed that a thorough study of the cytology of the two parent species or varieties, and if possible of the "intersexual" hybrids, was very desirable.

During our investigation a number of other points of interest appeared, some of which are dealt with here and to some we hope to return in a later paper. External events have at present interfered with the continuation of the work, and we have not been able to obtain intersexes for examination, so that the present paper makes no pretence of elucidating the questions in regard to sex which led to our beginning the work. The cytology of the spermatogenesis, however, is so remarkable, and in some respects so unlike anything known in other forms, that we think it advisable to publish our results on the pure species for their own sake and to return to the subject in a later paper if we are able to do so.

2. METHODS.

The method adopted for breeding the lice required was that used by Nuttall. The lice (P. corporis) were kept in small, round cardboard boxes, in the bottom of which a hole about $\frac{3}{4}$ in. in diameter was punched and covered with fine muslin. Each box contains a piece of cloth for the lice to

rest on. The boxes were kept in an incubator at about 30° C., and were fastened with an elastic band on the wrist or leg for half an hour twice each day. The lice feed readily through the muslin. A number of boxes can be worn at once without interfering with the activities of the wearer, and for most people, unless there are very many lice in a box, hardly any discomfort is caused. P. capitis feeds less readily, and the boxes containing it should be worn on the person continuously.

For preserving testes or ovaries for cytological examination, it was found that the simplest method was to place the louse in a drop of water on a glass slide under a mounted lens, behead it with a needle, and while holding the thorax by means of a needle with one hand, cut off the tip of the abdomen with a scalpel or sharp needle with the other. By light pressure on the abdomen from before backwards with a needle it was then quite easy to squeeze out the whole contents, and to place the whole mass, from which the testes or ovaries usually protrude conspicuously, in fixative. In male nymphs of Stage I, even immediately after hatching, the testes are almost always clearly visible by this method as paired bodies like two pears placed with their thicker ends together; in female nymphs of Stages I and II the ovaries are less easily seen, but can usually be found without much difficulty with a little teasing. It is thus possible to record the sex of nymphs, especially of Stages II and III, quickly and accurately by this method, without spending the time and trouble needed for rearing them to a stage when the external sexual characters become visible.

Several fixatives were tried, but with testes much the best results were obtained by Flemming's strong solution or modifications of it. Experiments were also made of dissecting the lice in water, salt, or in Ringer's solution, and in Flemming, but in general water gave the best results. For the study of chromosomes most of our material was fixed in Flemming without modification, but since in some stages the chromosomes are closely packed together and difficult to see

separately, we tried Flemming with twice and three times the normal proportion of acetic acid, in the hope of spreading them by causing the cytoplasm to swell ; in some cases we got clearer figures of chromosomes and of axial filaments of developing spermatozoa by this method. For the study of mitochondria (which, however, are often well shown in testes of Pediculus fixed with normal Flemming) we used chiefly Flemming without any acetic ("F.w.a."), either of normal strength or diluted with water. Flemming without acetic, diluted with three times its volume of water ("F.w.a. $\frac{1}{4}$") gave particularly good results. We also tried the method recommended by Champy of treating material fixed in " F.w.a." (full strength) followed by 3 per cent. potassium bichromate for forty-eight hours at 50° C., without, however, finding any improvement. The ovaries of older females were well fixed with such sublimate mixtures as that of Gilson. Most of our sections were stained on the slide by Heidenhain's iron-hæmatoxylin method. Several other stains and combinations of stains were used for comparison, usually without any great success in elucidating points which the hæmatoxylin preparations left obscure. Some of these will be referred to below.

The description which follows is taken from our preparations of P. corporis, but we have found no essential differences between P. corporis and P. capitis.

3. Spermatogenesis.

a. General Outline.

The testes are already well developed, though small, in nymphs immediately after they hatch from the egg.   At this stage, and throughout Stage I—i. e. before the first moult—they contain only spermatogonia.[1] These cells are arranged in groups or follicles, an arrangement which is not very

[1] The question whether the cells at this stage are spermatogonia or should properly be called primary spermatocytes will be discussed below.

evident among the resting cells, but is clearly seen when all the cells in one follicle are in division while the surrounding cells all have resting nuclei. They probably divide a number of times, for in young Stage II nymphs, i. e. after the first moult, the testes contain many more cells, some of which are now beginning to increase in size and are becoming spermatocytes. This takes place chiefly at the thicker end of the testis, nearest the vas deferens, and even in Stage III, spermatogonia like those of Stage I remain at the distal end and for a considerable distance along the sides of the testis, while the interior is filled with spermatocytes and later stages.

During the growth-phase the spermatocyte enlarges considerably and the mitochondria become conspicuous; there is at this stage a large nucleolus. At the close of the growth-phase the mitochondria coalesce to form a conspicuous " mitosome," which usually appears vacuolated. At the same time a deeply-staining round body appears near the nucleus, which we call the acroblast, since we believe it is concerned with the formation of the acrosome of the spermatozoon; it will be discussed more fully below.

After the growth-phase the spermatocytes undergo a division which is remarkable in two respects. In the first place, it is the only spermatocyte division, for as far as we have been able to discover no second division occurs ; and in the second, it is strikingly unequal as regards the cytoplasm of the cell, resulting in the separation of a nucleus with a small amount of cytoplasm from a relatively large cell which retains the whole mitochondrial body and the " acroblast." The division is in most respects much like the single spermatocyte division of the honey bee, and, as in the bee, the small cell degenerates without developing further, while the large cell is converted into a spermatozoon. The most remarkable feature of the later stages is that the centrosome of the spermatid, as the large cell must now be called, is paired, and from each centrosome an axial filament grows out, with the result that in its latest stages each spermatid has two very

conspicuous axial filaments.   The behaviour of the mitosome
and "acroblast" will be described below.

B. The Nucleus and Chromosomes.

In cells of the body of both males and females, and in
certain cells of the testes which are conspicuously larger than
the spermatogonia, and which, from their occurring singly
between the groups of spermatogonia, are doubtless follicle
cells, the chromosome number is twelve.   We have found
this number so frequently that there can be no doubt of it
(Pl. 16, figs. 1–4).   In the cells which we call spermatogonia,
however, mitotic figures show equally clearly six chromo-
somes.   In equatorial plate and anaphase figures the number
six is always perfectly clear (Pl. 16, figs. 6, 8, 9), though in a
few figures in quite young nymphs (Stage I, first or second
day after hatching from the egg) the chromosomes are visibly
double with an arrangement of the pairs which does not
suggest a longitudinal split preparatory to division (Pl. 16,
fig. 7).   And in prophases, especially in Stage I nymphs, but
also in the later stages, this doubleness of some of the
chromosomes is more conspicuous (Pl. 16, fig. 5).   In no male
germ-cell, from nymphs a few hours old onwards, have we
ever found twelve distinct chromosomes such as occur in the
somatic cells; the only possible exception is in the rather
large single cells mentioned above, and we are confident that
these are follicle cells and not cells of the "germ-track."
The question therefore arises whether the numerous testi-
cular cells which in the equatorial plate and later stages of
mitosis clearly have half the somatic chromosome number, are
indeed spermatogonia, or whether they are spermatocytes;
and if they are spermatogonia, what is the explanation of
their possessing the haploid (reduced) chromosome number?
In favour of their being spermatocytes there is, in addition
to their number of chromosomes, the fact that after the
growth phase there is only one cell division—the unequal
division referred to in Section A above.   These facts caused

us at first to regard these cells as primary spermatocytes, but we have been compelled to conclude that they are really spermatogonia by the fact, which appears to us indubitable, that they divide repeatedly before the growth phase commences.   Quite typical mitotic figures with six chromosomes occur in the testis of Stage I nymphs not yet a day old, at a time when the testes are very small and contain comparatively few cells.   Several days later, in young Stage II nymphs, the testes are much larger, but there has been no increase in the size of most of the cells, and the number of the cells is greatly increased.   The small cells at the apex of these older testes, and on into Stage III, have exactly similar mitotic figures with six chromosomes.   It seems evident, therefore, that these cells are true spermatogonia, but that for some reason they contain the haploid chromosome number.   On the analogy of the Hymenoptera, we supposed that possibly the male louse developed from an unfertilised egg, and had the haploid number throughout its germ-track, but, as will be seen from the section on the maturation of the egg, this is almost certainly not so.   Eggs laid by virgin females never develop, and no evidence was obtained from sections of eggs preserved shortly after being laid that the egg-nucleus ever segmented without conjugating with a sperm nucleus.   The only suggestion that we have to offer, therefore, is that in Pediculus there is precocious pairing of the chromosomes, and that the six chromosomes seen in spermatogonial mitoses are each double or bivalent consisting of a pair of univalents united together.   If the pairing were not yet quite complete, this would account for the prophase figures with double chromosomes, or sometimes with apparently as many as seven or eight, to which we have already referred.

In this connection we would refer to the work of Wilke (12) on the spermatogenesis of Hydrometra.   His paper is somewhat obscure, but he writes quite definitely that there are eleven chromosomes in the spermatogonial mitoses, and twelve, which he interprets as ten bivalents and two univalents, in those of the primary spermatocytes.   If he is

correct, it would appear that in this genus also the spermato-
gonial chromosomes are bivalent.[1]

The resting spermatogonial nucleus is large in comparison
with the small volume of the cytoplasm, and contains a con-
spicuous nucleolus (Pl. 16, fig. 5). In Stage II (after the first
moult) groups of these cells in the proximal and middle part
of the testis begin to increase in size, and the mitochondria
become visible in the cell body (Pl. 16, figs. 15–17). The
description of these, and of the "acroblast," which appears
rather later, is postponed to a later section. When the cells
have attained their full size the nucleolus disappears, and six
chromosomes appear in the nucleus. With Breinl's stain
(safranin and methylene blue; for method see 'Ann. Trop.
Med. and Parasitology,' vol. i, p. 470) the nucleolus stains blue,
suggesting that it consists of chromatin, which is possibly
used up in the formation of the chromosomes; we have not
followed the process in detail. In the majority of cells at
this stage, which must now be called spermatocytes, six
chromosomes are visible in the nucleus, differing among
themselves somewhat in size, but hardly enough to recognise
them as individuals; one is sometimes recognisably smaller
than the rest. In some groups of cells, however, it is quite
evident that each of these chromosomes is double (Pl. 16,
fig. 10), composed of two short rods which may appear paired
either end-to-end or side-to-side. The rods are so short in
comparison with their width that observations on the mode
of pairing can hardly be trustworthy, but it is fairly evident,
from the degree of separation of the members of each pair,
that the doubleness is due to pairing and not to splitting.
The nuclear membrane now disappears, and a spindle is
formed between two conspicuous centrosomes lying near the
margins of the cell. A striking peculiarity of the spindle at
this stage is that it is always very eccentric, it lies at one
side of the cell, so that the whole of it is contained in

[1] In a species of Gerris, a related genus of Hydrometridæ, I find that
the spermatogonia contain twenty-four and the spermatocytes twelve
chromosomes, which suggests that possibly Wilke is mistaken.—L. D.

considerably less than one hemisphere (Pl. 16, fig. 11), the
other side of the cell being largely occupied by the conspicuous
mitochondrial body, which by this time is very compact.
The chromosomes became arranged on the spindle in an
equatorial plate, and, in some cases at least, the mitosis has
reached the early anaphase stage, when the form of the cell
undergoes a striking change, which from the comparative
rarity of the stages must take place very quickly. The
spindle elongates considerably, and one pole of it, with its
centrosome, is carried outwards at the tip of a finger-like
process, which in the late anaphase projects to a distance of
half the diameter of the cell or even more (Pl. 16, figs. 12,
13, 14).   Occasionally it is possible to count six chromosomes
travelling toward each pole, but in the late anaphase the
spindle is extremely narrow, and the chromosomes become so
bunched together that it is rarely possible to see them
separately, especially in the part of the spindle contained in
the narrow process. The six chromosomes at what may be
called the proximal end of the spindle (the end directed
away from the finger-like process) travel to the pole and
form a vesicular nucleus in the neighbourhood of the mito-
some and "acroblast," which have remained stationary in
the body of the cell. The chromosomes which have entered
the finger-like process pass outwards to its tip and there
form a compact nucleus or chromatin mass. The protoplasm
of the process then seems to round itself off around this
nucleus into a little mass resembling a polar body, which at
first is connected with the body of the cell by a narrow neck
(Pl. 16, fig. 30), but soon becomes completely separate (Pl. 16,
figs. 28, 29). These little masses remain for a considerable
time in the neighbourhood of the spermatids and around
the heads of the developing spermatozoa; they undergo
no further development and seem finally to disintegrate.

As will be seen from Pl. 16, figs. 11–14, the whole division has
a close resemblance to the single nuclear division in the
spermatocytes of the hive bee, as described by Meves (**10**)
and by Mark and Copeland (**8**), or to the first spermatocyte

division of Aphids as described by Morgan (**11**) and by
von Baehr (**1**). In the case of the Aphids a teleological
explanation (if the words may be used) of the unequal
division can be given, for the small spermatocyte lacks a sex
chromosome which is present in the large one. In the bee,
however, no adequate explanation of the phenomena has ever
been given, and we are equally at a loss to account for the
similar behaviour of the spermatocytes of the louse. And in
one respect the louse is still more anomalous, for the bee
resembles all other Hymenoptera in having only one spermato-
cyte division, which is doubtless equational, in correspondence
with the fact that its germ-cells have throughout the haploid
number of chromosomes, since the male is developed from an
unfertilised egg. There is no reason, however, for believing
that this is true of the louse, and though the absence of a
second spermatocyte division is doubtless correlated with the
fact that the spermatogonia have six instead of twelve
chromosomes, there is still considerable obscurity as to the
cause of there being only one spermatocyte division. For it
seems certain that the apparently haploid chromosome
number of the spermatogonia is due to precocious pairing
of the chromosomes, perhaps comparable with the paired
arrangement of homologous chromosomes characteristic of the
Diptera (cf. Metz, **9**), and this is confirmed by the existence
of conspicuously double chromosomes in the spermatocyte
prophases of well-preserved testes (Pl. 16, fig. 10). If,
therefore, the general belief is justified that the heterotype
division is, so to speak, a special incident, due to the pairing
of the chromosomes, intercalated into the middle of an
ordinary mitosis which is completed in the succeeding homo-
type division, it should be expected that a second division
would be required in the louse, as in all other known cases
of sexually produced organisms, to separate the halves of
longitudinally split univalents. In the louse we have found
no evidence of the existence of this split, and it would appear
to be unique in having a heterotype division which is not
followed by a homotype.

Immediately after the spermatocyte division described above, the large cells (as distinguished from the minute polar cells), which must now be called spermatids, begin the final stage of their development—their transformation into spermatozoa. The nucleus has at first a considerable size and contains masses of chromatin, and sometimes traces of a reticulum are visible (Pl. 16, fig. 26), but as development proceeds the nucleus shrinks and the chromatin becomes aggregated into a mass in the centre (Pl. 16, figs. 27–30). The later stages of the nucleus, when the cell is elongating to form the spermatozoon, are somewhat obscure, and will be most conveniently considered in the section on spermiogenesis (" spermateleosis ") after the behaviour of the mitochondria, centrosomes and " acroblast " have been described.

c. Cytoplasmic Structures and Later Development of the Spermatozoa.

We have not succeeded in demonstrating mitochondria with any certainty in the spermatogonia. In some cells of this stage small stained granules appear in the neighbourhood of the nucleus, but they are not constantly seen, and we are not certain that when they are present they may not be artefacts. When the growth-phase has begun, however, a cloud of staining granules appears at one side of the nucleus, which is now very excentric and often almost at one margin of the cell (Pl. 16, figs. 15, 16). Almost from its first appearance this mass of granules is vacuolated, giving a foamlike appearance, and a little later the outer vesicles enlarge or coalesce to form prominent vacuoles enclosing a more solid, deeply-staining mass. At this stage the appearance of the mitochrondrial body is that which Gatenby (3) ascribes to faulty fixation (Pl. 16, figs. 18–21), and we are not prepared to deny categorically that this may not be the explanation of the appearances seen in our preparations. Before assuming that this is so, however, it must be noticed that several facts point to their being a true representation of the natural

condition. Firstly, cells at this stage fixed in F.w.a. $\frac{1}{4}$ (dilute Flemming without acetic) are no less vacuolated than those fixed in normal Flemming. Secondly, in the later stages of the spermatid we have beautiful figures of the mitosome, showing a definite and fairly constant structure which we think can hardly be artificial, and these occur in the same testes which have the vacuolated mitochondrial mass in the spermatocytes. Thirdly, the vacuoles of the early stages appear to play a definite part in giving origin to the structure of the mitosome of the spermatid. Just as Gatenby in the Lepidopteran testes found vesicles coalescing to give rise to his "mitochondrial spireme," so we find spermatocyte vacuoles apparently coalescing to give rise to the structure of the mitosome (Pl. 16, figs. 21–24). We think it not improbable that our mitochondrial figures in the younger cells may have undergone some distortion in fixation, but we hesitate to ascribe the whole structure as we see it simply to the action of the fixative. We find that in some cells preserved in normal Flemming, and in all in Flemming with two or three times the normal amount of acetic acid, the mitochondria are more or less completely dissolved, but we do not find large differences in structure in so far as structure is visible.

As the spermatocyte approaches division, the deeply staining substance of the vacuolated mass of mitochondria becomes aggregated as a more compact mass, surrounded by a few large vacuoles which separate it from the surrounding cytoplasm, and these vacuoles appear to coalesce into two, which enclose a central mass between them (Pl. 16, figs. 20–24). The central mass itself does not appear to be homogeneous, but to contain a space, or sometimes several spaces, enclosing a substance which stains less deeply. In the spermatocyte until after the unequal division the whole body is not far from spherical, consisting of two hemispherical vacuoles enclosing between them a somewhat ovoid, deeply-staining, more or less hollow mass.

Before this stage is reached there has appeared near the nucleus a spherical, deeply-staining body about the size of

the nucleolus (Pl. 16, figs. 10, 14, 25–29).   Since it appears at a time not far removed from that at which the nucleolus disappears, we thought at first that it might possibly be the nucleolus which had somehow emerged from the nucleus into the cytoplasm.   Further examination, however, showed that the extra-nuclear body co-exists for a time with the nucleolus, and sections stained with Breinl show that the nucleolus stains blue, and the extra-nuclear body, which we provisionally call by the name "acroblast," adopted from Gatenby, stains purplish-red.   In some spermatocytes the acroblast may be double about the time of its first appearance, but it is always a single spherical body later.   It is probably constantly present in suitably preserved cells, but it is not always visible; we suspect that when it cannot be seen it may have been dissolved by reagents.   The mitochondrial mass also shows extraordinary variability in staining capacity, even in adjacent cells in the same section, and it seems probable that the visibility or apparent absence of the "acroblast" may be due to the same cause, whatever this may be. We have been unable to determine with certainty either the origin or nature of the "acroblast," but on the analogy of the bodies in Lepidoptera, which Gatenby calls by the same name, we suspect that it belongs to the Golgi apparatus. Attempts to prove this by Kopsch's method (prolonged fixation in osmic acid without staining) have, however, failed to confirm this belief ; in sections of testes treated in this way we have failed to find the body.   Its subsequent history, so far as we have been able to follow it, will be described below in conjunction with that of the spermatid nucleus.

The chief remaining structure of interest in the developing spermatozoon is the centrosome apparatus and axial filaments. There is nothing peculiar about the centrosomes of the spermatocyte, except the extrusion of one of them with the small "polar" cell in the unequal division.   Very shortly after that division, however, when the cell is just beginning to elongate, it may be seen that the centrosome lying between the nucleus and the mitosome is double (Pl. 16, figs. 24, 25).

This stage is difficult to find, for very shortly after the division, from each of the halves of the double centrosome an axial filament begins to grow out (Pl. 16, fig. 26) ; the two filaments grow back in close connection with the outer sheath of the mitosome, and appear to have thickenings (" distal centrosomes ") at their free ends.   When they reach the posterior margin of the cell these distal centrosomes usually remain on the margin (Pl. 16, figs. 27, 29, 31) ; they sometimes appear to be carried out a short distance on thin processes like the antennæ of a butterfly (Pl. 16, fig. 30), but this appearance is not constant. A continuation of the axial filaments grows out beyond them as two thin flagella-like threads, or, probably through their being twisted together, as what looks like one thread (Pl. 16, figs. 29–31).

It can sometimes be seen that one of the axial filaments is thicker than the other, but in consequence of their close proximity to the edge of the mitosome this is not always easy to make out (Pl. 16, fig. 27). Transverse sections of elongating spermatids show very clearly the paired axial filaments running along the mitosome, but completely outside it, and the same is seen in transverse sections of the tails of well-advanced spermatozoa. There can be little doubt that the paired centrosomes and axial filaments are connected with the absence of a second spermatocyte division.

The mitosome in the spermatid has the same general structure as was described above in the mature spermatocyte, except that the whole body now appears bilaterally symmetrical, consisting of two outer symmetrically placed vacuoles enclosing a central, more deeply staining mass.   This central mass often contains a cavity or vacuoles ; many preparations (e. g. Pl. 16, fig. 32) give the impression that it is divided into two in the plane of junction of the two outer vacuoles, but it is possible that this appearance is due to the line of junction of the outer vacuoles running over its surface. Sometimes the inner mass clearly contains several distinct cavities (Pl. 16, fig. 25). For a time these structures gave us the impression that there was a spirally-coiled plate lying in

a non-staining space (cf. Pl. 16, fig. 28), but closer examina-
tion leads us to believe that this appearance is due to looking
at the body obliquely, and that nothing corresponding with a
"mitochondrial spireme" exists in Pediculus. As the cell
elongates, the mitosome becomes drawn out in length (Pl. 16,
figs. 33–35), and at a rather later stage the greater part of it
is contained in a conspicuous swelling towards the posterior
end of the long cell (Pl. 16, figs. 35, 36, 48a). After this
stage the cells become so attenuated that we do not feel con-
fident in tracing the fate of the mitosome further.

There remains to be described the development of the
anterior end of the spermatid—the nucleus and acrosome.
In the early spermatid the nucleus lies at what will be the
anterior end, the two centrosomes just behind it, and the
acroblast is behind or on one side of these (Pl. 16, figs.
33–37). For some time no important change takes place,
but when the cell is considerably elongated the acroblast
comes into contact with the nucleus, which is now small and
spherical, and usually stained evenly and faintly or with a
more deeply staining posterior area next to the centrosome.
The acroblast now becomes pressed against the nuclear
membrane and begins to spread out over one side of it like
a small cap, and then grows forward over the nucleus until
its anterior end projects in front of it (Pl. 16, figs. 38–41).
In successful Breinl preparations at this stage the nucleus is
stained blue and the acroblast cap red. Most of the staining
substance of the acroblast (as seen in iron-hæmatoxylin
preparations, which show the detail much better than those
stained in any other way) is concentrated in the forward
extension, but there is generally a band of more deeply
stained substance running back like a ridge along the middle
line of the cap to another mass at the posterior end close to the
centrosomes. The whole structure is so small and the parts
in such intimate contact that it is difficult to make out with
certainty how much is of nuclear origin and how much is
derived from the acroblast, but in Breinl preparations of this
stage the cap appears red and most of the posterior part blue,

which confirms our conclusion, derived from hæmatoxylin preparations which show more detail, that at least the whole of the projecting portion in front of the nucleus is derived from the acroblast. Whether some part of the acroblast remains at the posterior end is less certain, but as Breinl preparations often have a red region just in front of the centrosomes, it seems probable that this is so. The acroblast, however, does not seem to give rise to the deeply stained oval body which lies apparently within the nucleus at the posterior end of more elongated heads (Pl. 16, figs. 42–48); this body does not stain red with Breinl, and we incline to the belief that it is part of the nucleus. At this stage the whole head is becoming narrowed and elongated, and the acroblast projects in front of it as a point, widening behind to enclose the nucleus; it appears to have a shape something like that of the prow of a canoe, or like the cap of an Eschscholtzia bud when it is split along one side as the flower begins to open. There can be no doubt that the anterior projection becomes the acrosome, but as the head elongates the whole structure becomes so narrow and evenly stained—black with hæmatoxylin and uniform bright red with Breinl—that the further differentiation is impossible to follow.

D. Pediculus capitis.

The description given above is taken from our preparations of P. corporis, though a few of the figures with which it is illustrated, especially of the head of the developing spermatozoon, are drawn from sections of P. capitis. We have found no constant difference between the two forms as far as cytology is concerned; as far as we have been able to see, the chromosomes, mitochondrial body, acroblast, centrosomes and axial filaments are alike in both. The capitis sections gave the best figures of developing spermatozoa, but it is probable that this is an accidental effect of technique, and not due to any real distinction. Our work, therefore, as far as it has progressed, has not thrown any light on the cause of the

production of the intersexes found in the hybrids bred by Bacot.

4. Observations on the Development, Maturation and Fertilisation of the Egg.

We add here a few observations on oögenesis which are admittedly extremely incomplete, and we give them only to draw attention to several points of interest which we hope others may take up if we are unable to continue the work. We have not succeeded in finding any indubitable oögonial divisions, but equatorial plates of mitoses in follicle cells of the more advanced egg-tubes show twelve chromosomes very clearly. In young oöcytes six threads may be counted in the nucleus in the synapsis stage.

As the egg-tubes develop they become divided into follicles enclosing the growing oöcytes alternating with nutritive chambers. The latter consist of cells with large irregular nuclei which appear to be degenerating; each nutritive chamber is anterior to the oöcyte in connection with it. As the oöcyte enlarges, a deeply staining rod appears in the neighbourhood of the nucleus, and becomes a very conspicuous body in the older oöcytes (Text-fig. 1). It is of considerable length, often somewhat bent, and appears to consist of a hard substance, for in transverse section the portion in the thickness of the section is often displaced to some extent by the razor. Very often it has a small clear space around it, as if it had contracted in fixation and had left a space between it and the yolky cytoplasm of the egg. Sometimes in older oöcytes we have found a shorter accessory rod near the large one, and in young oöcytes we have seen two very small rods apparently shortly after their first appearance, but most commonly only one is present. Although we have spent considerable time in searching for its origin, we have not yet got any clue as to how the rod arises, and its fate is no less obscure. The egg in the oviduct becomes enclosed in a hard shell, which cannot be cut with a microtome, and in eggs

immediately after they are laid, from which it is not difficult
to peel off the shell, we have failed to find the rod.   We

TEXT-FIG. 1.

Growing oöcyte of Pediculus corporis enclosed in its follicle;
   nutritive cells at the anterior end.   The deeply staining rod is seen
   close to the nucleus.

believe that the nature and origin of this rod would well
repay fuller investigation.

    A no less enigmatic structure is present in the egg at the

time of laying. At its posterior end there is a mass of irregularly coiled bodies, which stain rather deeply with hæmatoxylin, lying at the edge of the yolk. Freshly laid eggs, even from the same female, differ widely from one another in the thickness of the layer of finely granular protoplasm which surrounds the inner yolky mass. In some eggs this layer is quite thick, in others very thin, and there is similar variation in the size and position of the " posterior mass," as we call for convenience this group of coiled granular substance. In eggs with a thick outer layer the posterior mass usually lies in it and does not project into the yolk, but when the outer layer is thin, it is usually invaginated into the central yolky substance as a sort of vesicle, filled with the " posterior mass." We have not attempted to follow the development of the egg beyond the early segmentation stages, so we can say nothing about the fate of the posterior mass ; we understand that work on the subject is in progress in other hands. We would only point out that in appearance the mass has a very close resemblance to the mass of supposed symbiotes figured by Buchner in the egg of certain Hemiptera (2).

We have, however, attempted to trace its origin, but, as in the case of the rod mentioned above, have not yet succeeded in doing so. The mass somewhat resembles the degenerating nuclei of the nutritive chamber of the ovarian oöcyte, and we suspect that it may be derived from it. The difficulty of this suggestion is that the nutritive chamber is at the anterior pole of the egg, at the opposite end from the " posterior mass." Until we have made satisfactory preparations of the full-grown oöcyte in the oviduct, in which we have not yet been successful, the matter must be left in suspense.

It is by no means easy to obtain satisfactory preparations of the maturation-divisions of the egg of Pediculus. The shell is extremely tough and resistant, and though it can be removed from eggs preserved in Gilson's or Carnoy's fixatives and subsequently placed in alcohol, we have not succeeded in shelling unfixed eggs, and in eggs preserved in their shells the fixation is not very good. Gilson's solution gives much better

results than anything else we have tried.   We find that in
order to remove the shell it is necessary that the egg should
be treated with solutions containing chloroform, but it is
possible to use acetic alcohol or acetic alcohol sublimate
without chloroform as a fixative, and to treat the egg with a
mixture of alcohol and chloroform afterwards, in order to
loosen the shell.

Although we have cut sections of a large number of eggs
preserved within an hour or two hours after they were laid,
we have found no case of the actual polar mitotic figures.
Some eggs appear to begin their development in the oviduct,
and to be already segmenting when they are laid, but others
are laid before the conjugation of egg and sperm nuclei takes
place.   We have obtained several figures showing two or three
polar nuclei near the edge of the egg (the outer polar nucleus
is very small and apparently soon degenerates), and some of
these eggs show either both the egg-nucleus and sperm-nucleus,
sometimes with the tail of the spermatozoon some distance away,
or if the egg is a little older, the first or second segmentation
divisions may be found.   In later eggs the polar nuclei have
disappeared.   A curious feature of the later segmentation
divisions is that many of the nuclei appear to be double, or
sometimes triple—that is to say they often consist of two or
sometimes three vesicles in contact, each containing a nuclear
reticulum.   We have found no evidence that the egg may ever
develop without conjugation of egg- and sperm-nuclei.

5. Breeding Experiments.

Since Hindle (**6, 6a**) found that the offspring of a large
proportion of individual pairs of lice were either all male or all
female, we hoped that by fixing the eggs of such pairs during
the maturation stages we might obtain evidence with regard
to the cytological basis of sex-determination in the louse.
We therefore isolated a number of virgin females and paired
them each with a single male.   Altogether eighteen such
pairings were made, in addition to several individuals which
were paired with a second mate after the first mate had died.

The original pairings are lettered A–S in the following table, and when the female of a pair was used again with a fresh mate, the second pair is represented with the same letter with a subscript 2, thus C_2.   The number of eggs laid was nearly always counted, and sometimes they were reared in separate successive batches as in mating J, to see whether the sex-ratio varied between the earlier and later eggs.   The sex of the offspring was determined by dissection, usually in Stage II.   We have to record our indebtedness to Dr. Keilin for kindly doing some of these dissections while one of us was away from Cambridge and the other temporarily prevented by indisposition from taking charge of the experiments.

DATE OF MATINGS.

Pair.	Number of eggs.	Offspring and sex.
A	12	6 ♂ 6 ♀
B	49	28 ♂ 20 ♀
B_2 ♀ of B, fresh ♂	19	12 ♂ 1? ♀
C	49	27 ♂ 18 ♀
C_2 ♀ of C, fresh ♂	20	13 ♂ 4 ♀
D	Infertile	
D_2 ♀ of D, fresh ♂	24	22 ♂ 1 ♀
E	Infertile	
E_2	10	— 3 ♀
F	40	24 ♂ 15 ♀
G=fresh ♀ × ♂ used in A	48	23 ♂ 21 ♀
G_2 ♀ of G, fresh ♂	15	6 ♂ 5 ♀
H	Infertile	
J first batch	19	13 ♂ 5 ♀
second batch	Not counted	6 ♂ 23 ♀
third batch	..	12 ♂ 12 ♀
K	64	♂ ♀ not counted
L	28	13 ♂ 4 ♀
M	14	5 ♂ 6 ♀
N	39	♂ ♀ not counted
O	28	♂ ♀ ..
P	Not counted	5 ♂ 10 ♀
Q	30	♂ ♀ not counted
R	14	♂ ♀ ,.
S	18	♂ ♀ ..

Total: 31 ♂ 40 ♀ (for J batches)

As will be seen from the table, there was no mating which certainly gave offspring of one sex only. B_2 gave 12 ♂ and one female recorded as doubtful out of 19 eggs, and D_2 22 ♂ and 1 ♀ out of 24 eggs. The family E_2, which contained only three females, is not large enough to be significant. The fact that in mating J there was such large variation in sex-ratio in different batches (in batch 2, 14 females were dissected in succession and then a group of males found), suggests that possibly the existence of apparently unisexual broods may be due rather to chance fluctuations than to a physiological difference in the parents. Hindle kept no record of the number of eggs laid, so did not know the mortality rate, but in our experiments in most families the sex of nearly all the eggs was recorded, except in the last few matings, in which the conduct of the experiments was accidentally interfered with.

6. SUMMARY.

The main facts of the spermatogenesis have been given in Section 3 A above. The most important points are as follows:

(1) The somatic chromosome number of both sexes is twelve, but spermatogonial mitotic figures show only six. There is some evidence that these are double, and we ascribe the existence of the apparently haploid number in the spermatogonia to premature pairing.

(2) There is only one spermatocyte division, which is extremely unequal, leading to the separation of a minute polar-body-like cell which degenerates.

(3) The centrosome of the spermatid is double, and from each half an axial filament grows out, so that the developing spermatozoa have two conspicuous axial filaments.

(4) The development of the mitochondrial body is described.

(5) The acrosome is derived from a deeply staining body which appears in the spermatocyte and becomes applied to the nucleus of the spermatid like a cap.

(6) The existence of a deeply stained rod, of unknown origin and fate, in the growing oöcyte, and of a posterior

mass of stained granules in the mature egg, is shortly referred to. The egg nucleus undergoes two polar divisions, and fertilisation appears to be essential if the egg is to develop.

(7) Breeding experiments did not confirm Hindle's observation that broods consisting only of males or only of females are frequent. Some broods with great preponderance of one or the other sex were obtained.

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